March 8, 1948.

Dr. W. S. Stone,
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Austin, Texas.

Dear Dr. Stone,

I was glad to hear that your group has so much as run through the first steps in recombination experiments with K-12 mutants. So far as I am aware, this is the first independent "confirmation", although the mutants have been widely distributed for a long time. I might say that results come so fast with coli, that there is a lot of unpublished data on segregations of all kinds of mutants. If anything like that can be of help to you, please let me know.

As to your manuscript. I wish that this kind of data had been published along with your first report, because it certainly makes for a very convinc case. I can see no pronounced objections to the conclusion that irradiated or peroxide-treated broth probably induces "mutations". Of course without genetic analysis, it is not certain (however likely), as you pointed out, that these are qualitative changes in individual units, but the same could be said for almost any mutagenic effect on a microorganism. I have a few comments, however, which you may be interested to hear:

1. From Table 3, it appears that most of the inhibitory effect of irradiate broth involves the first two hours after inoculation. Table 1 would have been the more cogent if it had involved comparisons of 0 and 2 h Table 3, however, adequately duplicates the results.

2. Your method of testing for mannitol-fermentation is not clear. Do you use mannitol-agar, and count yellow colonies, or do you pick colonies to separate tubes. I am not sure that it makes any difference.

3. Frankly, the most objectionable aspect of using drug-resistance mutations, as I am sure you will agree, is the variability in expression of the character which is indicated in Table 1. You can never be sure tha you are counting all your mutants, be they treated or not. On the other hand, have you tested a great many colonies appearing on drug-plates to be sure that only mutants are counted?

4. During the inhibited period of growth in irradiated broth, do you get a close correspondence between your plate counts and the optical densit

of the cultures. I ask this because it is a nuclear population in which mutations presumably occur, while your plate count may measure something else.

Have you considered using phage-resistance? That has worked, of course, very well with E. coli, but I understand that irradiated broth has no effect on coli mutations.

Although these questions could be clarified, I do not think they are critical enough to invalidate your general conclusions. As to your interpretation, I am not sure that I follow you. The Demerec' delayed effect can still be satisfactorily explained as a segregation of nuclei or chromatids or whatnot, not to mention phenotypic delay. Do you still adhere to the statement that specific mutations can be induced by irradiati particular components? It seemed to me that this was the strongest support of the "assimilation" hypothesis.

Yours sincerely,

Joshua Lederberg.